Energy Biosciences Institute

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IN THIS ISSUE

K.C. TING, AT RIGHT, AND BP CEO TONY HAYWARD DISCUSS CROP MONITORING SYSTEMS NOW IN PLACE AT THE EBI ENERGY FARM.

Modeling and Monitoring Key to Peak Feedstock Production

University of Illinois Professor K. C. Ting says the road to successful liquid biofuels will travel through two value chain subsystems—the biomass production system and the biomass-to-fuel conversion process. He and his team of investigators, collaborators, postdocs and graduate students in the Energy Biosciences Institute are focusing on a "holistic" approach to that first subsystem which will optimize yield, efficiency, cost-effectiveness, and environmental benefits.

"Engineering Solutions for Biomass Feedstock Production" is the title of both Ting's EBI program and a story he authored in the April-May issue of Resource, the membership publication of the American Society of Agricultural and Biological Engineers (ASABE). Subsequent presentations by members of Ting's program at ASABE's annual international meeting in Reno, NV, in June expanded on the elements of their studies.

Central to their investigations is the development, led by Yogendra Shastri, of a computer model called "BioFeed," which explores the strategic and management-level issues in biomass feedstock production, such as refinery capacity, farm machine selection, and transportation fleet scheduling.

"The goal of this research," said Ting, "is to develop a modeling and systems analysis framework that allows us to explore issues that will impact the efficiency improvement of bioenergy feedstock production."

This farm-to-refinery analysis focuses on five interrelated tasks: pre-harvest energy crop monitoring, harvesting of energy crops, transportation and storage of biomass, and systems informatics and analysis (the data analysis that evaluates system performance). Results will emerge in the forms of enabling technologies, machinery design and prototypes, informational databases, and decision support tools.
Shastri and his team applied the BioFeed model to a hypothetical scenario of switchgrass production in southern Illinois. The results they reported at the ASABE meeting illustrated that transportation is the most expensive operation, packing is the most energy-intensive task, the often-ignored storage step is relatively expensive, and farm machine selections and storage decisions are largely dependent upon farm size.

The model also identifies the sustainable biorefinery capacity for a given collection area so that a year-round supply of biomass can be assured.

Next, the BioFeed model will be applied to different bioenergy crops and geographical regions to study the impact of distributed farming and collection, the importance of various storage options, and the impacts of technology development.

A series of sophisticated monitoring devices has been developed and activated in the fields at the EBI Energy

(cont. on page 8)

Two-Stage Pretreatment Process Enhances Cellulose Digestibility

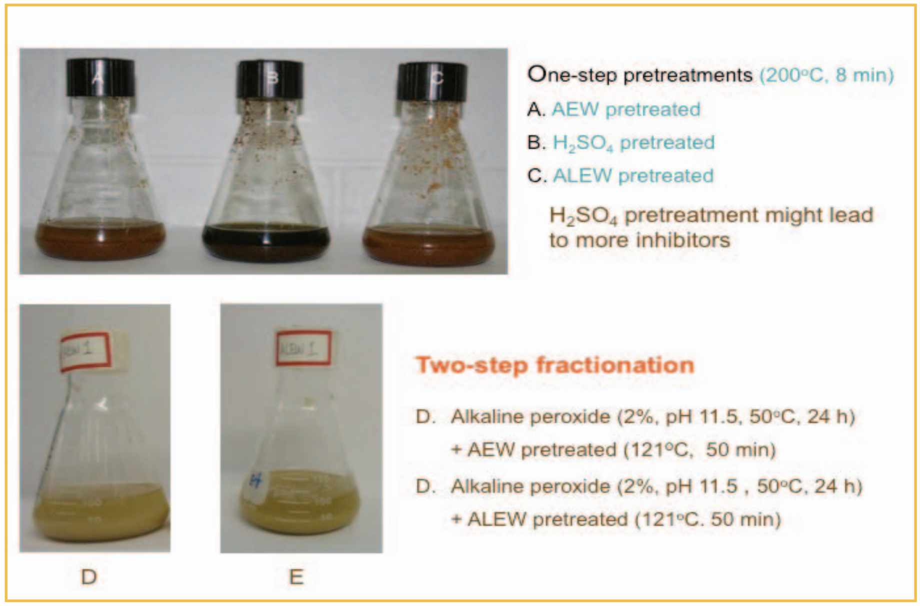
A major challenge in reducing the expense and efficiency of processing biofuels lies in unlocking the protective structures in plants that bind the carbohydrates, from which sugars are drawn for enzymes to attack in fermentation. Nearly two-thirds of production costs for cellulosic ethanol is in the processing, which includes pretreatment of the feedstock.

Now a team of scientists from the Energy Biosciences Institute has discovered a two-stage pretreatment method that shows promise in enhancing cellulose digestibility, thus maximizing sugar potential while minimizing toxic inhibitory products. The technique may be more cost-effective than most biomass pretreatment methods used today, and more environmentally friendly.

The results of the work, conducted by EBI principal investigator and University of Illinois food engineering professor Hao Feng and visiting Chinese research scientists Bin Wang and Xiaojuan Wang, have been published

HAO FENG, AT RIGHT, WITH GRADUATE STUDENT XIAOJUAN WANG

in the journal Bioresource Technology. The research was applied to a promising perennial grass called *Miscanthus*, whose fast growth, high yields, long productive lifetime and minimal water requirements make it an ideal bio-



The photos show *Miscanthus* samples after the one-step (top) and two-step pretreatment. The 1% acid pretreatment (B) produced the darkest *Miscanthus* slurry. The alkaline-electrolized water samples (A and C) are also relatively dark. The two-step pretreated *Miscanthus* slurries (bottom) had a light color, from which chromatography analysis did not detect ethanol fermentation inhibitors.

fuel feedstock. However, *Miscanthus* generally requires harsh treatment conditions to deconstruct the cellulose, hemicellulose and lignin found in the plant cell walls. These tough substances give rigidity and form to plants.

Switching from the conventional one-step pretreatment methods, Feng and his colleagues first treated the *Miscanthus* with an alkaline peroxide solution at different concentrations, which partially removed the hemicellulose (63 percent) and lignin (64 percent). The second treatment of the residual cellulose-rich solids used acidic or alkaline electrolyzed water to deconstruct crystalline cellulose fibers for hydrolysis by enzymes into sugars.

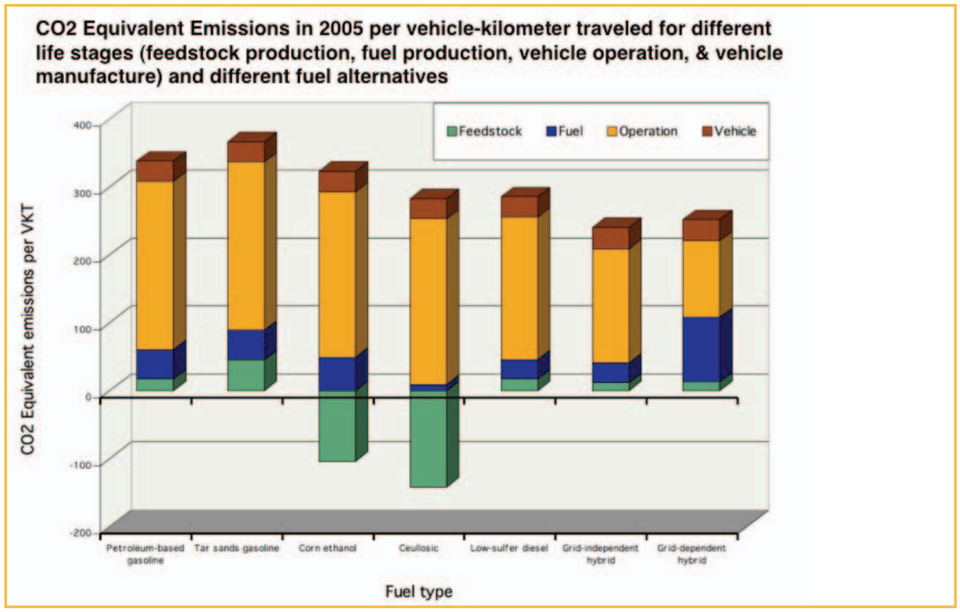
Pretreatments at high temperatures, especially those using acids or water, generally degrade the cellulose, hemicellulose and lignin and form inhibitory products, which are toxic to fermenting organisms. The use of the two-stage method negates the need to detoxify biomass prior to the enzymatic and fermentation stages of fuel production.

"If one can remove hemicellulose and lignin up front, a value-added stream can be produced and the subsequent pretreatment can be conducted under mild conditions," the authors stated. "The sugar yield should be improved and the inhibitor generation could be minimized." They noted that, after the second pretreatment step at a relatively low temperature, up to 95 percent of the cellulose was digested through hydrolysis.

The experiments offer promise that deconstruction of biofuel feedstocks like *Miscanthus* can be accomplished faster and using less energy, which converts to a less costly operation.

Feng said the group is now concentrating on optimizing the process conditions and recovering the solubilized hemicellulose and lignin for possible additional conversion to fermentable sugars. The hemicellulose-rich portion of *Miscanthus* is also being evaluated by other EBI program teams to perform enzymatic hydrolysis with a novel enzyme cocktail and fermentation with genetically engineered yeast.

'Hidden' Costs of Energy Could Drop With Advanced Biofuels



This chart from the academy report shows CO_2 equivalent emissions for different life cycles and a set of vehicle/fuel alternatives. Cellulosic is lowest among the fuel alternatives.

A National Research Council panel, on which EBI investigator Tom McKone served, has estimated in a report that the "hidden" costs of energy production and use can be far more extensive than those reflected in market prices of coal, oil and other energy sources. Those damages the committee was able to quantify were an estimated $120 billion in the U.S. in 2005, a number that reflects primarily health damages from air pollution associated with electricity generation and motor vehicle transportation.

The EBI is investigating advanced transportation fuels, specifically non-food cellulosic biofuel, which the committee report said will have lower damages than most other vehicle fuel options when commercially available. In addition, "achieving significant reductions in greenhouse gas (GHG) emissions by 2030 will likely also require breakthrough technologies, such as cost-effective carbon capture and storage or conversion of advanced biofuels," the report summary says.

Requested by Congress, the blue-ribbon panel's report assesses what economists call external effects caused by various energy sources over their entire life cycle—for example, not only the pollution generated when gasoline is used to run a car but also the pollution created by extracting and refining oil and transporting fuel to gas stations and manufacturing the vehicle. Because these effects are not reflected in energy prices, government, businesses and consumers may not realize the full impact of their choices. When such market failures occur, a case can be made for government interventions—such as regulations, taxes or tradable permits—to address these external costs, the report says.

Nineteen leading scientists and academicians were chosen for the study based upon their expertise and experience, and the final report was subject to external peer review. McKone, an expert on environmental risk assessment who is senior scientist at Lawrence Berkeley National Laboratory as well as adjunct professor in UC Berkeley's School of Public Health, collaborated with other members to develop the committee's overall life-cycle framework and apply it to transportation fuels. He is co-principal investigator of the EBI program studying the environmental and economic life cycle impacts of alternative fuels.

"Perhaps our most significant contribution is to map out some of the complex upstream links in the energy supply chain in a way that allows consumers and policy makers to better consider ways to reduce the impacts of electricity and transportation services," McKone said. "For example, many aspects of transportation systems and transportation fuels depend on electrical energy or process heat. How one produces that electricity and/or energy has a strong impact on the overall health and environmental costs attributable to each vehicle-kilometer of transportation service."

The committee focused on monetizing the damage of major air pollutants—sulfur dioxide, nitrogen oxides, ozone, and particulate matter—on human health, grain crops and timber yields, buildings, and recreation. When possible, it estimated both what the damages were in 2005 (the latest year for which data were available) and what they are likely to be in 2030, assuming current policies continue and new policies already slated for implementation are put in place.

The committee also separately derived a range of values for damages from climate change; the wide range of possibilities for these damages made it impossible to develop precise estimates of cost. However, all model results available to the committee indicate that climate-related damages caused by each ton of CO_2 emissions will be far worse in 2030 than now; even if the total amount of annual emissions remains steady, the damages caused by each ton would increase 50 percent to 80 percent.

The report notes that transportation, which today relies almost exclusively on oil, accounts for nearly 30 percent of U.S. energy demand. It evaluated a variety of fuels, including ethanol derived from corn. "E85 from herba-

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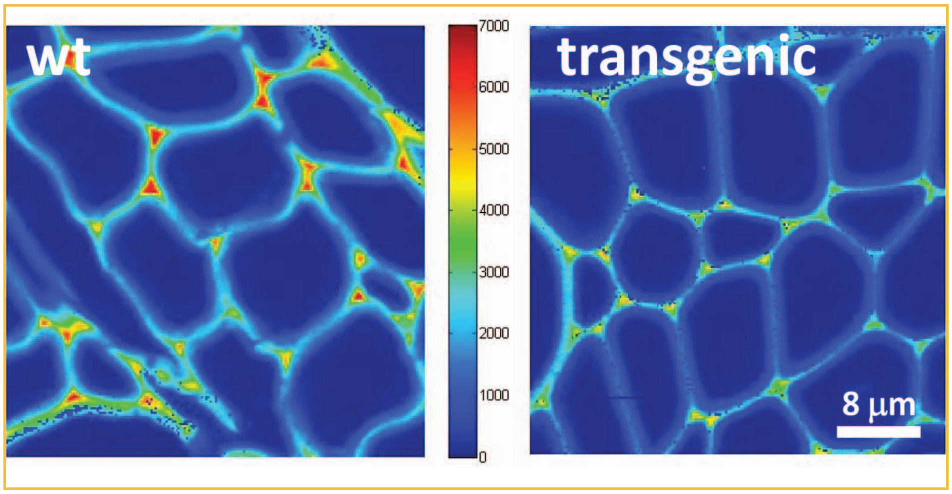
Microscope Technique Assesses Lignin Reduction in Plant Cell Walls



Raman images of lignin in the poplar wood cell walls -- spatial distributions of the Raman signal intensity of lignin in a wild-type (left) and a transgenic sample

Much of the work being pursued by lab investigators at the Energy Biosciences Institute is focused on methodologies and technologies that will enable the economic and efficient breakdown of cellulose into fermentable sugars.

One of the most problematic components to deconstruct in lignocellulosic biomass is lignin, which can comprise up to about 25 percent of the plant. It is a complex substance in the cell walls of vascular plants, essential for the stiffness and strength of stem and root, and which also waterproofs the plant and helps protect it against pathogens. But it impedes saccharification and thus becomes a limiting factor in post-harvest lignocellulosic breakdown.

The amount of lignin in wood can be reduced through transgenic alteration of the plant, and such low-lignin wood offers potential for improved biomass conversion into fermentable sugars. A recent experiment by EBI spectro-microscopists has for the first time visualized the outcome of such transgenic alteration by acquiring and comparing chemical images of wild-type and lignin-deficient transgenic poplar wood.

"Plant cell walls have a highly complex hierarchical structure," said UC Berkeley postdoctoral researcher Martin Schmidt, primary author of a scientific paper published as a "Rapid Communication" in Planta, an on-line journal. "Their detailed structure remains to be fully elucidated, but such detailed knowledge is required to understand the chemical and physical obstacles to breakdown."

The EBI has two programs dedicated to unscrambling the architecture of the cell wall. Schmidt is with one of those teams, headed by Lawrence Berkeley National Laboratory bioengineer Paul Adams, which is dedicated to the chemical imaging of plant biomass using Raman microscopy—a technique that relies on inelastic or Raman scattering of monochromatic light, like that from a laser, where the shift in the energy of the laser photons gives information about molecular vibrations of the sample. "Raman microscopy can afford non-destructive and comparatively inexpensive measurement with minimal sample preparation," Schmidt said. "In terms of studying plant traits that may affect lignocellulose conversion efficiency, this technique holds great potential for dynamic chemical in situ (in its natural setting) imaging."

In collaboration with Vincent Chiang and co-workers from North Carolina State University, the EBI team investigated stem wood from wild type and transgenic *Populus trichocarpa*, also known as black cottonwood or California poplar. The unique qualities of the confocal Raman microscope allowed for the viewing and comparing of the lignin vibrational bands within the samples—all without having to stain or label the tissues—with a lateral spatial resolution of about 300 nanometers.

Lignin has become a key plant breeding and genetic engineering target for researchers outside of the EBI, in view of improving deconstruction pretreatment methods and cell wall conversion. "This analytical tool," said Jim Schuck, corresponding author and a staff scientist at Berkeley Lab's Molecular Foundry, "can be invaluable as rapid characterization of plant cell walls becomes increasingly important for evaluating feedstock traits, such as composition and structure of compounds like lignin."

The EBI experiment found differences in lignin content, localization, and chemical composition in the cell walls. The transgenic wood showed significantly reduced lignin intensities over the wild type. Such reduction may alleviate recalcitrance toward sugar conversion; the team will next focus on evaluating the effect of different degrees of lignification on pretreatment and enzymatic hydrolysis efficiency.

The paper, "Label-free in situ Imaging of Lignification in the Cell Wall of Low Lignin transgenic *Populus trichocarpa*," can be read at http://springerlink.com/content/e84633213l23g535/

Other EBI authors listed on the paper are Adams, Manfred Auer, Pradeep Perera, Purbasha Sakar, Elena Bosneaga, and Andrew Carroll.

Researcher Consensus: Biofuels Beneficial if Produced Properly

Some of the international leading lights in biofuel research—including Energy Biosciences Institute Director Chris Somerville—gathered in Princeton one day in June of 2008 to exchange views about the sustainability of biofuels, food and the environment. They came from a range of backgrounds and perspectives, and the debate was lively. But consensus emerged in the form of this statement:

"Society cannot afford to miss out on the global greenhouse gas emission reductions and the local environmental and societal benefits when biofuels are done right. However, society also cannot accept the undesirable impacts of biofuels done wrong."

A report on their discussion appeared in the July 16, 2009 issue of the journal Science under the title "Beneficial Biofuels—the Food, Energy and Environment Trilemma."

"The world needs to replace fossil fuels with renewable energy, but recent findings (such as the recognition of the potential importance of effects from indirect land use) have thrown the emerging biofuels industry into a quandary," said the University of Minnesota ecologist David Tilman, lead author of the paper. "We met to seek solutions. We found that the next generation of biofuels can be highly beneficial if produced properly."

The workshop was funded by BP and Ford through the Carbon Mitigation Initiative at Princeton University.

To balance biofuel production, food security and emissions reduction, the authors concluded that the global biofuels industry must focus on five major sources of renewable biomass: perennial plants grown on degraded lands abandoned from agricultural use, crop residues, sustainably harvested wood and forest residues, double crops and mixed cropping systems, and municipal and industrial wastes.

"The five biomass sources…in combination with large reductions in fuel demand could produce enough biofuels to meet a substantial portion of future energy demand for transportation," the paper stated. It also noted "several wrong options," including clearing land of native ecosystems and displacing food crop lands.

"Dramatic improvements in policy and technology are needed to reconfigure agriculture and land use to gracefully meet global demand for both food and biofuel feedstocks," it said. "Three steps should be taken: meaningful science-based environmental safeguards should be adopted, a robust biofuels industry should be enabled, and those who have invested in first-generation biofuels should have a viable path forward."

In closing, the paper called for "a new collaboration between environmentalists, economists, technologists, the agriculture community, engaged citizens, and governments around the world."

In addition to Somerville and Tilman, scientists taking part in the discussion included Minnesota's Jonathan Foley and Jason Hill; Princeton's Robert Socolow, Eric Larson, Stephen Pacala, Tim Searchinger and Robert Williams; Dartmouth's Lee Lynd; and MIT's John Reilly.

EBI RESEARCHERS ARE TESTING THE VIABILITY OF A NUMBER OF DIFFERENT POTENTIAL BIOFUEL FEEDSTOCKS ON THE ENERGY FARM.

BP Leadership Shares Belief in Promise of Alternative Fuels

In separate addresses by BP's chief executive officer and its president of BP Biofuels North America, the energy company's leadership has reiterated its firm commitment to the development of alternative energy sources, especially cellulosic biofuels.

CEO Tony Hayward told an audience at MIT in October, "We believe that biofuels will become very significant businesses in the coming years and that they could make up almost 10 percent of global transport fuel by 2030 and potentially as much as 20 percent of the U.S. gasoline pool." North American Biofuels President Susan Ellerbusch, in testimony before Congress, cited the Energy Biosciences Institute (EBI) public-private partnership as an indication that "BP supports a robust biofuels industry where many players will bring forward a variety of technology and commercial solutions."

And the Chief Executive of BP Biofuels, Phil New, told the World Ethanol Conference in Paris that the company expects to break ground on a commercial production facility for making cellulosic ethanol in Florida and is currently building a wheat-based ethanol plant near Hull in eastern England. The latter will be retrofit to produce biobutanol in a few years, he said.

Hayward's talk, given a few days after he visited EBI's Energy Farm at the University of Illinois, emphasized the need to concentrate on biofuels "that provide high energy and real environmental benefits without damaging nutrition or biodiversity." He cited ethanol from sugar cane (like that being produced in Brazil), biobutanol, and lignocellulosic fuels as prime candidates.

"Ethanol from energy grasses is potentially a very good answer to the challenges of access, sustainability and security," he noted. "It offers high energy yields. It does not compete with food. It provides a literally home-grown substitute for imported oil. And it has the potential not simply to cut emissions but to be a net absorber of carbon."

A joint venture between BP and Verenium

Hayward stressed the importance of innovation for the future. "The really interesting things happen at the borders where different disciplines meet. In particular, there is real scope to apply some of the enabling technologies that have made such dramatic progress over the past decade." For example, he said, biotechnology is being supported by BP in the EBI. "This not only provides the chance to develop advances in biofuels, but also to explore how biotech might open up new possibilities from exploration to carbon sequestration," he added.

He urged governments to create a "road-map" and set the framework within which energy markets can deliver. "These are not issues on which we have endless time to deliberate," he cautioned. "It matters what we do over the next 25 years."

Ellerbusch addressed the House Agriculture Subcommittee on Conservation, Credit, Energy and Research during hearings on U.S. energy policy. While urging long-term government investment in biofuels research to mitigate the risks being taken on by BP and other private companies, she said BP is prioritizing what it identifies as the strongest biofuel options for increasing energy security, reducing greenhouse gas emissions and supporting sustainable agriculture. These include cellulosic biofuels in the U.S., biobutanol that can be deployed in existing and new ethanol facilities in Europe, and sugarcane-based biofuels in Brazil.

She cited BP's $500 million investment in the EBI, as well as the BP partnership with Verenium Corporation to build the first commercial-scale cellulosic biofuel facility in the U.S., as indicators of the company's seriousness in pursuing the next generation of biofuels.

New followed both talks with a presentation in which he said that, to compete with fossil fuels in the future, biofuels will have to live without subsidies and keep costs within $1 a gallon on a volume basis. "If you can't chin that bar, you're not going to be able to compete in the long run," he said, stressing that sugar cane currently is the most cost-efficient. He also addressed the virtues of biobutanol as "an attractive alternative" to established ethanol since it allows higher blending levels.

"Biobutanol can provide a door through the blend wall which I would argue is the key structural barrier to the growth of this industry over the next five to 10 years," New said. Through a joint research venture in the United States, BP and Dupont are developing a process to produce biobutanol and will pilot the technology at a demonstration facility next year at the Hull plant in England.

New EBI Executive Committee Members

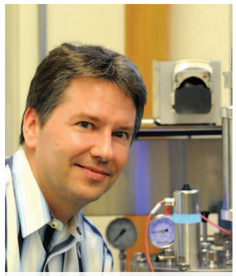
Professors Alex Bell and Doug Clark, who have appointments at both the University of California, Berkeley, and Lawrence Berkeley National Laboratory, University of Illinois environmental economist Madhu Khanna, and legal specialist Jody Endres have been added to the membership of the Energy Biosciences Institute Executive Committee.

The Committee is EBI's program management body, with Director Chris Somerville as chair. He, the deputy and associate directors, professors from the partner institutions and BP representatives comprise the committee membership. This panel proposes the annual strategic work plan, including priority research projects for institute funding, for approval by the Governance Board.


ALEX BELL

Bell is the Theodore Vermeulen Professor of Chemical Engineering at UC Berkeley and Faculty Senior Scientist at Berkeley Lab. He is a member of the National Academy of Engineering, a Fellow of the American Association for the Advancement of Science, and an elected member of the American Academy of Arts and Sciences. His EBI program is entitled "Biomass Pretreatment and Chemical Synthesis of Transportation Fuels."

Clark is Executive Associate Dean of the College of Chemical Engineering at UC Berkeley. As Professor of Chemical Engineering, he works in the field of biochemical engineering, with particular emphasis on enzyme technology and bioactive materials, extremophiles, cell culture, and metabolic flux analysis. He is a Fellow of the American Institute of Medical and Biomedical Engineers and the American Association for the Advancement of Science. Clark's EBI program is "Thermophilic Microbes and Enzymes for Biofuel Production."


DOUG CLARK

Endres is senior regulatory associate in the Law and Regulation Program at the Energy Biosciences Institute at the University of Illinois. A cum laude graduate of the University of Illinois School of Law, she has worked in a large litigation firm and has clerked for U.S. District Courts in Virginia and Arizona. She is a published author in the nation's top environmental law journals.


JODY ENDRES

Khanna is a professor in the Department of Agricultural and Consumer Economics at the University of Illinois, specializing in technology adoption and voluntary approaches to pollution control, welfare analysis of alternative policy instruments for environmental protection, and policies for biofuels and carbon sequestration. Her EBI program is entitled "Economic and Environmental Impacts of Biofuels: Implications for Land Use and Policy."


MADHU KHANNA

The full committee roster can be found on the EBI Web site (www.energybiosciencesinstitute.org).

'Hidden' Costs (CONT'D)

ceous feedstock (such as switchgrass) and corn stover (has) relatively lower damages than all the other options," it stated, and "corn-based ethanol, especially E85, has relatively higher damages than most other fuels…in large measure due to the higher level of emissions from the energy required to produce the feedstock and the fuel." And it concludes, "Some breakthrough technologies (e.g. cost-efficient conversion of advanced biofuels, cost-efficient carbon capture and storage and/or much greater use of renewable sources for electricity generation) will be needed to dramatically reduce transportation-related externalities."

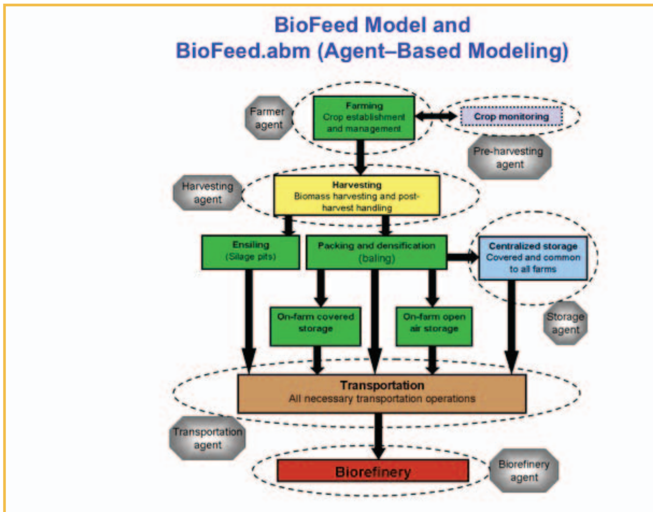
Additional research was urged to "improve understanding of the currently unquantifiable effects and potential damages, especially as they relate to biofuel (e.g. the effects on water resources and ecosystems)." The EBI has several projects under way looking at environmental impacts of biofuels, including water utilization.

The report also notes that the issue of indirect land use impacts, the subject of current debates about the merits of biofuels, needs closer scrutiny. "Regardless of whether this impact is regarded as an externality associated with U.S. or foreign biofuels production, it is important to obtain more empirical evidence about its magnitude and causes, as well as to improve the current suite of land use change models," the authors wrote.

EBI's McKone reflected on his year-long experience. "It was really an honor to serve on this committee and interact with some of the country's best environmental economists and health scientists to address such an important issue," he said. "It concerns me that people may see our report as the final word, but I hope they recognize that we have merely opened the door on this topic and raised a number of important questions that remain to be addressed."

Modeling and Monitoring (CONT'D)

Farm, 320 acres of biomass croplands near the Illinois campus. A tower remote sensing system was described in September at the 4th International Workshop on Bio-Robotics in Champaign, IL. The 38-meter-high tower incorporates a new method of image geo-referencing through the use of a 360-degree rotating camera with attached digital compass for orientation adjustment.



The BioFeed Model explores the strategic and management-level issues in biomass feedstock production, from the farm to the biorefinery.

The system has been developed to provide continuous near-real-time remote sensing images for crops in the experimental fields. The camera is controlled and the data stored by a computer attached to the tower. The spectral images have been evaluated with agronomic databases for estimating biomass feedstock over the growing season. Site-specific management using spatial information is required for locating the target point inside the field. In order to be able to target any specific point, the compass, through the interaction of magnetic field and gravity, controls the pitch and roll of the camera to focus on the geo-referenced point. Camera and compass calibration were delicate and essential to the quality of the images.

The pre-harvest energy crop monitoring task leader Lei Tian and his team members Tofael Ahamed, Yuliang Zhang, Yanshui Jiang, and Francisco Pinto developed the system.

The mechanical design for a second robotic data collection instrument, this one mobile on the ground, was described by Yonghua Xiong, Tian and Ahamed in the bio-robotics workshop. Their Reconfigurable Data Acquisition Vehicle – R-DAV – is a four-wheel-drive "agribot" intended for monitoring, samples acquisition, weed control, and chemical applications for crops. An adjustable clearance mechanism allows operation over different heights of plants in the fields. A changeable wheel gauge can adjust to narrow intra-row tracks.

For biofuel crops like *Miscanthus*, height and density of the plants requires a self-propelled field monitoring vehicle with high clearance. Such a device will eliminate the need for using large destructive tractors or human labor to collect things like crop growth status data, field samples, and images. The prototype, looking somewhat like a table on wheels, is being tested now. It consists of four legs, a chassis framework, operating arm, and a generator for power supply. Each leg has the capacity to lift, steer and drive.

A review paper submitted to a journal discussed current and future system informatics and analysis approaches, the basis for development of analysis tools that will allow information integration in order to model future systems.

"Unlike the petroleum industry, the biofuels industry is still in its infancy, and its value chain has not been thoroughly explored, let alone optimized," wrote lead author Shastri. "The analysis of this value chain should not only focus on the optimization of individual sectors, but also ensure the compatibility of various sectors in the value chain leading to overall (optimal) configuration" of an efficient feedstock production system.

The co-authors of that paper were Konstantinos Domdouzis, Ming-Che Hu, Alan Hansen, Luis Rodriguez, and Ting, all members of the Department of Agricultural and Biological Engineering at the University of Illinois.

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